GASEOUS DIMETHYL DIOXIRANE AS A DECONTAMINANT FOR SIMULANTS OF CHEMICAL & BIOLOGICAL WEAPONS

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Gaseous dimethyl dioxirane (DMDO) was evaluated as a decontaminant for simulants of chemical and biological warfare agents (CBW). Isolated DMDO was present as a liquid in CFC 113 or the fluorinated ether, Novec HFE 7100™ (3M) and was easily vaporized under reduced pressure. The H-agent simulant, thioanisole, and the G-agent simulants, paraoxon and diphenyl chlorophosphate (DPCP), were efficiently neutralized in less than an hour. In addition, *Bacillus subtilis* spores were completely deactivated by a 2-hour exposure to 64 μmoles of DMDO.

INTRODUCTION

Neutralization of chemical weapons typically entails incineration or destruction by caustic bleach or hydrogen peroxide [1, 2]. However, these solution-based decontaminants are not suitable for application to sensitive equipment. Despite the success of gaseous chlorine dioxide in deactivating *B. anthracis* spores during the recent decontamination of the Hart Building in Washington D. C., the search continues for a more convenient, faster-acting, and environmentally friendly decontaminant for biological and chemical weapons. Productive areas of study include enzymatic and chemical methods of destruction [1, 3, 4, 5, 6].

The addition of oxygen atoms to thioanisole simulates the oxidation of the H-agent, sulfur mustard by hydrogen peroxide in conjunction with the bicarbonate or molybdate ion [5]. If the reaction is limited to the addition of one oxygen atom, the mustard sulfoxide produced has lost the vesicant properties of the original compound. However, addition of a second oxygen atom produces the sulfone, a vesicant in its own right. In other degradation processes, oxidation of sulfur mustard by bleach is non-selective, yielding both sulfoxide and sulfone, whereas oxidation by hydrogen peroxide alone is very slow.

Aqueous dimethyl dioxirane (DMDO) has been used in the paper industry for chlorine-free bleaching by transferring oxygen atoms to sulfur, nitrogen, or carbon atoms found in cellulose products. Under conditions of excess phenyl methyl sulfide, Murray [7] found that DMDO in acetone produces phenyl methyl sulfoxide exclusively. It has been suggested that the volatility of DMDO presents the opportunity for having a gaseous oxidizing agent that might be capable of neutralizing sulfur mustard, as well as other CBW with electron-rich chemical bonds.

This study examines the effectiveness of gaseous DMDO in degrading thioanisole and the G-agent simulants, paraoxon and diphenyl chlorophosphate (DPCP). In addition, spores of the biological simulant of *B. anthracis (B. subtilis)* were exposed to gaseous DMDO and tested for their ability to regenerate under lab conditions.

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EXPERIMENTAL METHODS

1. MATERIALS

B. subtilis was from ATCC (Rockville, MD). Novec HFE 7100™ was the gift of 3M company (St. Paul, MN). CFC 113 (1,1,2-trichloro-1,2,2-trifluoroethane) was from Fisher (Pittsburgh, PA). All other chemicals and reagents were from Sigma-Aldrich (St. Louis, MO).

2. DMDO SYNTHESIS AND ENRICHMENT

Dimethyl dioxirane (DMDO) was prepared in acetone, as described by Adam and Hadjiarapoglou [8] and concentrated by a modification of the extraction reported by Gibert et al [9]. The concentration of DMDO was determined by titration with 0.02 N $Na_2S_2O_3$ in the presence of 1 M acetic acid and 0.07 M KI. Isolated DMDO was aliquoted into amber vials, sealed, and stored at –80° C until used.

3. EXPOSURE OF CHEMICAL SIMULANTS TO DMDO

Thioanisole, diphenyl chlorophosphate (DPCP) or paraoxon was pipetted onto 13 mm Teflon™ membrane filters (Pall Corp., Ann Arbor, Mich.) which were inserted into a Teflon™ tube inserted downstream of a 5 ml 2-neck reaction flask. The system was closed and a moderate vacuum was applied for 3 min. to ensure the absence of air. DMDO/HFE or HFE was added through a Teflon™ valve into the reaction flask, and the vapor generated was circulated with a peristaltic pump for the given time in the dark. A blank run was carried out by the same procedure, except that neither DMDO nor HFE was added.

4. ANALYSIS

DMDO was analyzed by UV absorbance at 305 nm against a solvent blank. The concentration was determined by a standard curve of DMDO in the same solvent. Chemical simulants and their oxidation products were were analyzed using HP GC model 5890 Series II, HP MS model 5971 and ChemStation© software (Hewlett Packard, Atlanta, GA). Standard curves of the pure compounds in acetonitrile were used to calculate the concentrations.

5. SPORE REGENERATION

Starter cultures were inoculated into nutrient broth plus yeast extract (NBY) (yeast extract added at 3 grams per liter nutrient broth media) and grown overnight at 35° C while shaking at 175 rpm. The starter culture was used to inoculate 10 liters of NBY broth and grown in a Microferm Fermentor (New Brunswick Scientific Co., Inc. Edison N.J. USA)at 37° C until the > 99% vegetative cells were spores as determined by microscopy. The cultures were harvested by centrifugation and suspended in sterile Milli-Q water. The spore suspension was pipetted onto a 7-mm glass fiber filter and inserted into the top of a sterile sample vial (8 ml). 500 µl of DMDO or CFC 113 were added to the vial along with a sterile stir bar and the top was immediately secured. The solutions were stirred slowly at room temperature for the given amount of time. The filters were removed to 10 ml of sterile water, vortexed, and plated onto LB agar for overnight incubation at 30° C. The colonies were counted in the usual manner.

RESULTS AND DISCUSSION

When DMDO and thioanisole were present in approximately equimolar amounts, the depletion of thioanisole was directly related to the disappearance of DMDO (Fig. 1). The data show that the sulfoxide is formed first, but after 30 minutes of exposure, an equivalent amount of the sulfone was present. The yield of products was 88%, 80%, 66% and 47% of the amount of thioanisole lost after 9, 15, 30 and 60 minutes, respectively. We were unable to determine why the product yield was not 100% of the reactant loss. The reason might possibly be due to the formation of a volatile product. The reaction chamber was rinsed with acetonitrile to dissolve any volatile material that might have condensed on the sides. However, if the unknown product were not soluble in acetonitrile, this would not be a productive

procedure. On the other hand, thioanisole or products could have reacted with some other component of the equipment, although care was taken to minimize the reactivity of the materials used in construction. We will examine this problem in future studies.

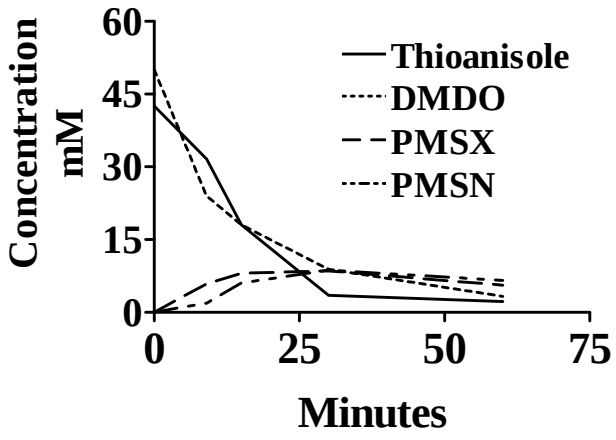

Fig. 1. Time course for the oxidation of thioanisole by gaseous DMDO.

DPCP is an organophosphate that simulates the nerve agents, sarin and soman, or GB and GD. Exposure of a 1.8-fold excess of DPCP to gaseous DMDO for 60 minutes resulted in the depletion of DPCP by 81% (Fig. 2). A slight shoulder at 265 nm is present in the product, but the amount of absorbance of the product is reduced and shifted to the left, indicating a change in the molecular structure. Product identification of this reaction is currently underway.

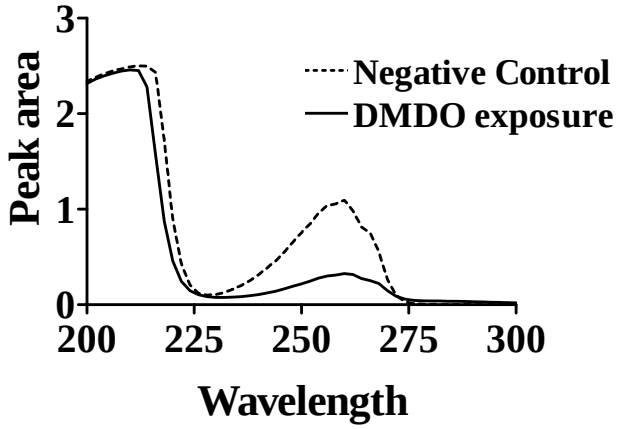

Fig. 2. UV spectra of the reaction between 96 μmoles of DPCP and 54 μmoles of gaseous DMDO. The negative control was CFC 113.

Paraoxon is also an organophosphate that serves as a simulant for the G-agents. Exposure of paraoxon to a 7.5-fold excess of gaseous DMDO for 60 minutes resulted in an 85.6% reduction of the parent compound. The data shown in Fig. 3 indicates that reaction begins within 3 minutes of exposure and progresses at a steady rate. Quantification of the reaction rate and identification of the products is currently in progress.

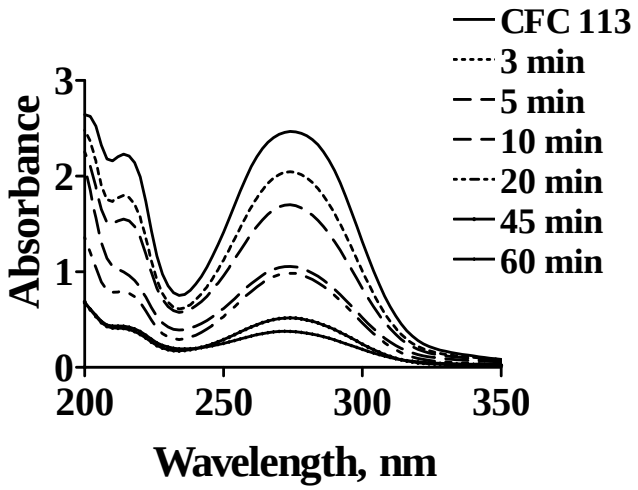

Fig. 3. UV spectra during oxidation of 0.4 μmoles of paraoxon with 3 μmoles of gaseous DMDO. Exposure to CFC 113 for 60 minutes is the negative control.

The bacterial spore is resistant to chemical attack due to metabolic dormancy and a complex protective covering composed of proteins, lipids, and carbohydrates [10]. Exposure of *B. subtilis* spores to gaseous DMDO for 120 minutes resulted in complete loss of the ability to regenerate, as shown in Fig. 4. The deactivation was apparent after 10 minutes of exposure, with a 98.5% decrease in viable cells, while a 60-minute exposure extended the damage to 99.7%. The mechanism of killing by gaseous DMDO is not known. However, Setlow and Young [11] showed that ozone appears to damage the inner membrane of the spore coat of *B. subtilis*. Our observations might possibly be attributed to oxidative damage of proteins, lipids, or other component of the spore coat.

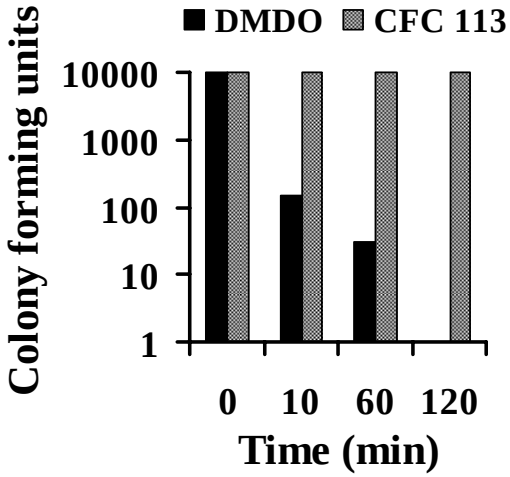

Fig. 4. Deactivation of *B. subtilis* spores by exposure to 64 μmoles of gaseous DMDO. CFC 113 is the

negative control.

CONCLUSIONS

Gaseous DMDO is an effective decontaminant for chemicals and biologicals that simulate weapons of mass destruction. Thioanisole was stoichiometrically oxidized the phenyl methyl sulfoxide and phenyl methyl sulfone within 30 minutes. Diphenyl chlorophosphate, in a 1.8-fold excess, was decreased by 81% during a 60 minute exposure to gaseous DMDO. Paraoxon was depleted in 60 minutes by a 7.5-fold excess of DMDO. Viable *B. subtilis* spores were reduced by 98.5% after exposure to 64 μmoles of DMDO vapor for 10 minutes. However, prolonged exposure for over 60 minutes was required for complete deactivation.

Dissolution of DMDO in a non-flammable solvent such as Novec 7100 HFE™ produces an oxidizing agent that proves to be effective in neutralizing simulants of both chemical and biological weapons. The solution is easily vaporized under reduced pressure, offering a convenient, non-corrosive and environmentally friendly alternative to current technology.

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